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Mitochondrial permeability transition in CNS trauma: Cause or effect of neuronal cell death?
AUTHOR: Sullivan P G (Reprint); Rabchevsky A G; Waldmeier P C; Springer J E
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2) **Long-term follow-up and complications after cardiac transplantation.**
Conrad S A; Chhabra A; Vay D
Willis Knighton-LSU Medical Center Heart and Lung Transplantation Center in Shreveport.
Journal of the Louisiana State Medical Society - official organ of the Louisiana State Medical Society (UNITED STATES) May **1993** , 145 (5) p217-20, 223-5, ISSN 0024-6921 Journal Code: 7505618
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3) **Prevention of cardiac hypertrophy in mice by calcineurin inhibition.**
Sussman M A; Lim H W; Gude N; Taigen T; Olson E N; Robbins J; Colbert M C ; Gualberto A; Wieczorek D F; Molkentin J D
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subcloned into *lacZ* (pPD21.28 and pPD16.01) or GFP (pPD95.75) reporter constructs (*26*) and assayed for β-galactosidase activity or fluorescence. One translational fusion construct in which 1.5 kbp of the *flp-1* upstream region and part of the coding region was fused in-frame to the *lacZ* gene and the remaining part of the coding region was placed after the stop codon of the *lacZ* gene was also made. Reporter constructs were co-injected with the *rol-6* pRF4 plasmid as described [C. C. Mello, J. M. Kramer, D. Stinchcomb, V. Ambros, *EMBO J.* **10**, 3959 (1991)]. All transgenic lines showed *flp-1* expression only in anterior head neurons, and for the most part, all transgenic lines had the same expression pattern.

11. K. Schinkmann and C. Li, *J. Comp. Neurol.* **315**, 1 (1992).
12. A. Rushforth, B. Saari, P. Anderson, *Mol. Cell. Biol.* **13**, 902 (1993); R. R. Zwaal, A. Broeks, J. van Meurs, J. T. M. Groenen, R. H. A. Plasterk, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 7431 (1993).
13. Populations of *flp-1*::Tc1 *(pk41)* animals were screened as described (*12*). The deletion amplification products were subcloned into the TA cloning vector (Invitrogen) and sequenced using the USB Sequenase 2.0 kit.
14. Southern (DNA) blot analysis was performed as described [J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) with a digoxygenin-labeled probe (Boerhinger Mannheim Genius System). Detection was performed with a chemiluminescent substrate (Boehringer Mannheim Genius System).
15. L. S. Nelson, data not shown.
16. S. Brenner, *Genetics* **77**, 71 (1984).
17. J. G. White, E. Southgate, J. N. Thomson, S. Brenner, *Philos. Trans. R. Soc. London Ser. B* **314**, 1 (1986).
18. C. M. Coburn and C. J. Bargmann, *Neuron* **17**, 695 (1996).
19. L. S. Nelson, unpublished observations.
20. J. M. Kaplan and H. R. Horvitz, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2227 (1993).
21. M. Chalfie and M. Au, *Science* **243**, 1027 (1989).
22. J. G. Culotti and R. L. Russell, *Genetics* **90**, 243 (1978).
23. S. Ward, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 817 (1973); D. B. Dusenbery, *J. Exp. Biol.* **188**, 41 (1974); C. I. Bargmann and H. R. Horvitz, *Neuron* **7**, 729 (1991).
24. L. S. Nelson and C. Li, data not shown.
25. The 6 kbp *flp-1* upstream region was amplified from wild-type genomic DNA and inserted into a construct with the *flp-1* coding region to yield LN/B3-SB. *flp-1(yn2); lin-15(n765ts)* animals were co-injected with LN/B3-SB (50 to 100 μg/ml) and JM24 (*lin-15*; 50 μg/ml). Transgenic animals (selected by rescue of the Lin-15 phenotype at 20°C) were scored for rescue of *flp-1* phenotypes as described.
26. A. Fire, S. White, D. Dixon, *Gene* **93**, 189 (1990).
27. E. G. Stringham, D. K. Dixon, D. Jones, E. P. M. Candido, *Mol. Biol. Cell.* **3**, 221 (1992).
28. A *flp-1* cDNA, 3-3F, was subcloned into the hsp vector pPD49.78 (*26*) to yield HSP-FLP1. *lin-15(n765ts)* animals were co-injected with HSP-FLP1 (100 μg/ml) and JM24 (*lin-15*; 50 μg/ml). Transgenic animals were selected by rescue of the Lin-15 phenotype at 20°C; the transgene was integrated by exposing transgenic animals to cobalt irradiation as described [J. C. Way, L. Wang, J.-Q. Run, A. Wang, *Genes Dev.* **5**, 2199 (1991)]. Two integrated lines (*ynIs9* and *ynIs10*) were established; both show the same phenotypes. *ynIs9* animals were crossed to *flp-1(yn2)* animals.
29. H. R. Horvitz, M. Chalfie, C. Trent, J. E. Sulston, P. D. Evans, *Science* **216**, 1012 (1982).
30. L. Ségalat, D. A. Elkes, J. M. Kaplan, *ibid.* **267**, 1648 (1995).
31. O. Chiba, K. Sasake, H. Higuchi, K. Takashima, *Neurosci. Res.* **15**, 255 (1992); K. Payza and H.-Y. T. Yang, *J. Neurochem.* **60**, 1894 (1993); G. J. Chin, K. Payza, D. A. Price, M. J. Greenberg, K. E. Doble, *Biol. Bull.* **187**, 185 (1994).
32. E. Linguegila, G. Champigny, M. Lazdunski, P. Barbry, *Nature* **378**, 730 (1995).
33. J. E. Mendel *et al.*, *Science* **267**, 1652 (1995); L. Brundage *et al.*, *Neuron* **16**, 999 (1996); R. R. Zwaal *et al.*, *Cell* **86**, 619 (1996).
34. L. Brundage, W. Chen, P. Sternberg, personal communication.
35. We thank R. H. A. Plasterk for generously screening for and providing the *flp-1*::Tc1 insertional strain NL242 *(pk41)*; L. Brundage, J. Mendel, P. Sternberg, J. Kaplan, L. Avery, and R. Korswagen for providing G protein–subunit mutants, advice on assays, and communication of unpublished results; H. R. Horvitz for providing mapping strains; A. Fire for providing microinjection vectors; J. Cherry and E. Widmaier for patiently providing help with the statistical analyses; S. Craven, H. Field, G. Jang, Y. Honzano, and C. Milliken for graciously providing technical assistance; T. Gilmore for comments on the manuscript; and members of the lab for helpful discussions. Supported in part by grants from NSF (IBN9320262) and NIH (AG00708) to C.L.

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Prevention of Cardiac Hypertrophy in Mice by Calcineurin Inhibition

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Hypertrophic cardiomyopathy (HCM) is an inherited form of heart disease that affects 1 in 500 individuals. Here it is shown that calcineurin, a calcium-regulated phosphatase, plays a critical role in the pathogenesis of HCM. Administration of the calcineurin inhibitors cyclosporin and FK506 prevented disease in mice that were genetically predisposed to develop HCM as a result of aberrant expression of tropomodulin, myosin light chain–2, or fetal β-tropomyosin in the heart. Cyclosporin had a similar effect in a rat model of pressure-overload hypertrophy. These results suggest that calcineurin inhibitors merit investigation as potential therapeutics for certain forms of human heart disease.

Heart failure is the major cause of disability and morbidity in the United States and affects about 700,000 individuals each year (*1*). Heart disease can arise from extrinsic stimuli such as hypertension or from intrinsic defects within the heart itself. HCM is the most common form of intrinsic heart disease and has been cited as the most frequent cause of sudden death in young people (*1*). HCM is defined by a generalized enlargement of the myocardium, but it can progress to heart dilation, functional insufficiency, and failure (*1*). Several intrinsic cardiomyopathies are caused by genetic mutations in contractile proteins that organize into repetitive units known as sarcomeres. Mutations have been identified in the genes encoding β-myosin heavy chain (MHC), cardiac troponin T, α-tropomyosin, myosin-binding protein C, myosin light chains (MLC), and cardiac α-actin (*2*). Normal sarcomeric function is associated with a basal concentration of intracellular calcium, which regulates contractility. It has been postulated that mutations in sarcomeric proteins lead to increases in intracellular calcium in order to maintain contractility and cardiac output (*3*). However, increases in basal calcium concentrations are also associated with cardiac hypertrophy.

We have shown that calcineurin, a calcium-regulated phosphatase, initiates cardiac hypertrophy when it is expressed in a constitutively active form in the heart of transgenic mice (*4*), suggesting a link between calcium concentration and a calcium-regulated signaling molecule in the heart. Calcineurin is activated by prolonged increases in basal concentrations of calcium, but not by transient calcium spikes associated with the activation of calcium-calmodulin–dependent kinase II (CaMKII) and mitogen-activated protein kinase (MAPK) (*5*). Together, these data suggest that calcineurin may play a pivotal role in signaling maladaptive hypertrophy in response to alterations in calcium handling in the heart.

To test this hypothesis, we treated four transgenic mouse models of cardiomyopathy and a rat model of pressure-overload hypertrophy with the calcineurin inhibitors cyclosporin (CsA) and FK506. We initially tested a mouse model of dilated cardiomyopathy caused by cardiac-specific overexpression of

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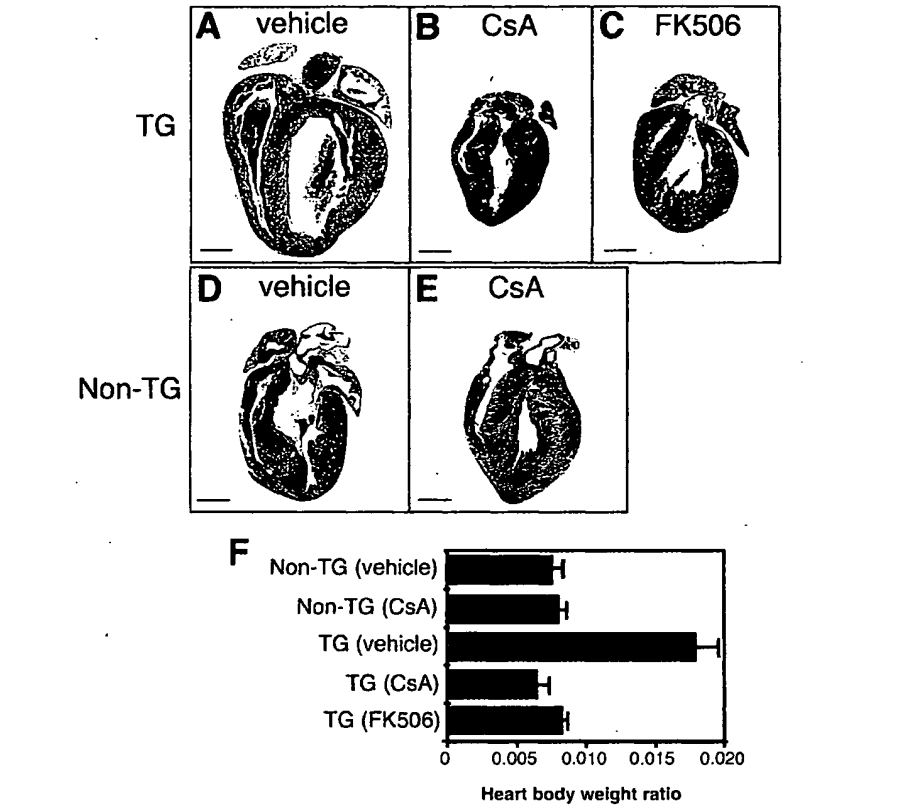
the actin-capping molecule tropomodulin (*6*). As determined by evaluation of multiple histologic sections, CsA and FK506 administration to tropomodulin-overexpressing transgenic (TOT) mice from day 9 after birth to day 24 prevented dilated cardiomyopathy in every animal treated (Fig. 1, A to C), but these inhibitors had no effect on normal growth-related developmental hypertrophy (*7*) (Fig. 1, D and E). Echocardiography was performed to substantiate this difference in morphology and to quantify ventricular dimensions in vivo. Mean measurements of left ventricular end-diastolic (LVED) dimensions were 3.24 ± 0.29 mm and 2.40 ± 0.04 mm in the vehicle-treated and CsA-treated groups, respectively. Left ventricular end-systolic (LVES) dimensions were 2.27 ± 0.28 mm and 1.72 ± 0.25 mm in the vehicle-treated and CsA-treated groups, respectively. Fractional shortening was not rescued, probably because the disease-causing stimulus (tropomodulin overexpression) had not been alleviated. The elevated heart/body weight ratio characteristic of TOT mice was restored to normal values in every mouse treated with CsA or FK506 (Fig. 1F).

The myofibrillar disarray that characterizes the hearts of TOT mice was also prevented by CsA (Fig. 1, I and K), although aberrant sarcomeric distribution of tropomodulin (*8*) in these hearts was still prevalent (Fig. 1L). Immunoblot analysis confirmed that the amount of expression of the tropomodulin transgene was not affected by CsA or FK506 (*9*). These results suggest that cardiac dilation in TOT mice is secondary to the activation of calcineurin. Lastly, histologic evaluation of hematoxylin and eosin (H&E)–stained heart sections revealed that CsA treatment prevented myopathic changes in TOT mouse hearts (Fig. 1, M to O).

The main biological effect of CsA and FK506 is the inhibition of calcineurin, but



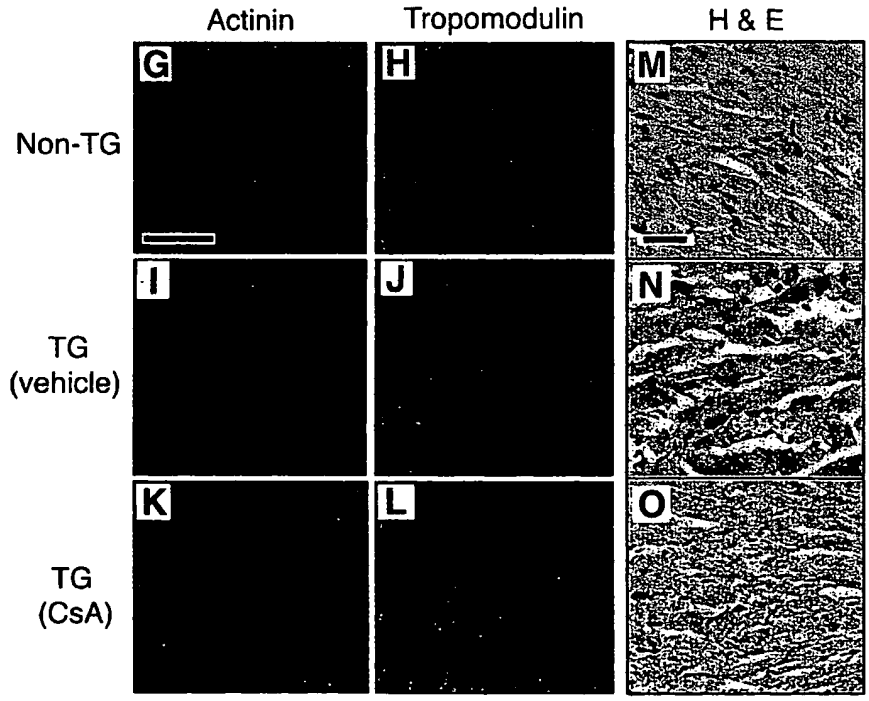


Fig. 1. Inhibition of calcineurin signaling prevents dilated cardiomyopathy in TOT transgenic (TG) mice. (**A**) H&E-stained histologic section of a TOT mouse heart, showing dilated cardiomyopathy by 24 days after birth. (**B** and **C**) Treatment of TOT mice with CsA (15 mg/kg twice a day) or FK506 (3 mg/kg twice a day) prevents dilated cardiomyopathy. (**D** and **E**) CsA administration to nontransgenic mice shows no inhibition of normal growth-related hypertrophy over this same treatment period. All histologic sections are presented with the atria on top and the right ventricle to the left. Scale bar, 1 mm. (**F**) Analysis of heart/body weight ratios demonstrates that CsA and FK506 inhibit increases in heart size in TOT mice, whereas growth-related cardiac hypertrophy is unaffected. Error bars are ±SEM of $n \geq 5$. (**G** to **L**) Confocal microscopy of heart sections from 24-day-old nontransgenic or TOT mice labeled with α-actinin antibody (red) or with tropomodulin antibody (green). In (G) and (H), nontransgenic hearts show ordered myofibrils with both α-actinin and tropomodulin antibody staining. Hearts from TOT mice show a loss of myofibril organization (I) and ectopic localization of tropomodulin (J), as described (*6*). CsA prevents myofibril disorganization in TOT hearts (K), despite tropomodulin overexpression (L). Scale bar, 10 μm. (**M** to **O**) H&E-stained histologic sections of a wild-type heart (M), a TOT heart (N), and a TOT heart treated with CsA (O). Scale bar, 20 μm.

each has been shown to have additional cellular effects (*10*). To confirm that the effect of these drugs on cardiomyopathy was mediated through calcineurin, we performed calcineurin enzymatic assays (*11*). Calcineurin activity in hearts from untreated TOT mice was 21.1 ± 3.5 pmol min^{-1} mg^{-1} protein, compared with 10.1 ± 2.9 pmol min^{-1} mg^{-1} for wild-type hearts and 10.9 ± 3.1 pmol min^{-1} mg^{-1} for TOT hearts treated with CsA. These data confirm the activation of calcineurin in TOT transgenic hearts. We also performed immunoblot assays on heart extracts from untreated TOT mice to look for changes in expression or activation of other signaling molecules that have been implicated in cardiac hypertrophy (*12*). We found no significant differences in the expression of Ras, extracellular-regulated kinase (ERK), or MAP kinase–2 (MEK2), or in the activation of MAPK and p38 (*9*).

The effect of calcineurin inhibitors was also tested in other mouse models of cardiomyopathy. Transgenic mice in which the MLC2v protein has been replaced with a mutant form of the protein that cannot be phosphorylated exhibit HCM as a result of inefficient sarcomeric cross-bridge cycling (*13*). CsA treatment of these transgenic mice prevented cardiac myopathic changes, as assessed by histologic analysis (*14*) (Fig. 2, A to C). CsA also decreased heart/body weight ratios in these transgenic mice, rescued individual myocyte hypertrophy as assessed by high-magnification histology (*9*), and inhibited the activation of fetal genes typically associated with cardiomyopathy, such as those encoding atrial natriuretic factor (ANF), skeletal α-actin, and β-MHC (Fig. 2J).

We next tested transgenic mice that overexpress β-tropomyosin in the heart, which results in cardiomyopathy and a defect in calcium handling (*15*). CsA treatment also prevented the development of dilated cardiomyopathy in these mice, as assessed by histologic analysis (*16*) (Fig. 2, D to F). Because elevated basal calcium concentrations are involved in the development of cardiomyopathy in β-tropomyosin transgenics, these results strengthen the conclusion that calcineurin mediates hypertrophic signaling in response to altered calcium concentrations.

The three mouse cardiomyopathic models described above have a common defect in contractility caused by perturbations in sarcomeric proteins. We also tested a transgenic mouse model in which hypertrophy is not directly related to sarcomeric dysfunction. These mice overexpress a constitutively active retinoic acid receptor (RAR) in the heart and are thought to develop cardiomyopathy as a result of alterations in the expression of retinoid receptor–dependent genes (*17*). Treatment of these mice with CsA did not inhibit the development of cardiac hypertrophy, as assessed by histologic analysis (Fig. 2, G to I) (*18*).

Our results suggest a model for the development of intrinsic heart disease in which compensatory increases in myocardial intracellular calcium directly activate calcineurin. Numerous studies have suggested that alterations in cardiac calcium handling occur in response to sarcomeric defects or hypertrophic stimuli (*3, 19*). One mechanism by which calcineurin signaling could initiate hypertrophy is through the transcription factor NFAT3, which is directly activated by calcineurin and translocated to the nucleus, where it activates a subset of hypertrophic response genes (*4*). A similar signaling pathway has been demonstrated in T cells (*20*).

The mouse models we used in our study mimic intrinsic forms of human heart disease that are caused by mutations in genes encoding cardiac contractile proteins. In most cases, human HCMs are dominant-negative mutations resulting in the production of a "poison peptide" that infiltrates and disrupts organized sarcomeres (*21*). Extrinsic forms of human heart disease, such as those caused by pressure overload, share common intracellular signaling mechanisms with intrinsic forms of cardiomyopathy. The pressure-overload stimulus initially increases ventricular wall

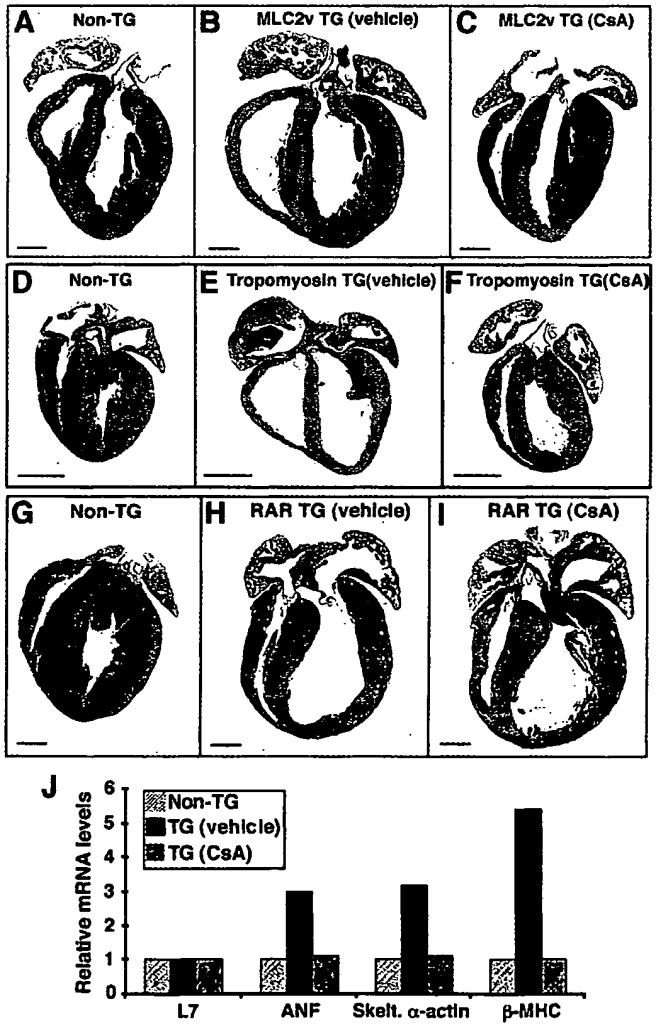


Fig. 2. (A to C) H&E-stained histologic heart sections show inhibition of HCM by CsA in 8-week-old MLC2v phosphorylation-negative transgenic mice (*14*). (D to F) CsA also prevents dilated ventricular myopathy in the hearts of β-tropomyosin–overexpressing mice (*16*). (G to I) CsA treatment did not prevent cardiac hypertrophy in RAR transgenic mice. Scale bars, 1 mm. (J) Semiquantitative (reverse transcription polymerase chain reaction) analysis of mRNA from wild-type and MLC2v transgenic hearts treated with vehicle or CsA. CsA treatment prevented the increase in amounts of ANF, skeletal α-actin, and β-MHC transcript, but had no effect on amounts of the ribosomal RNA marker L7. These data are consistent with RNA dot blot quantitation (*13*).

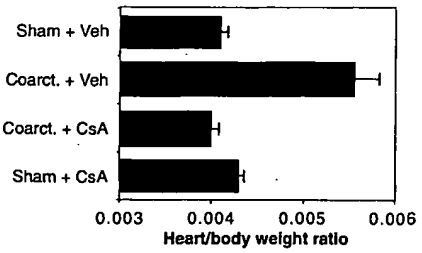


Fig. 3. CsA prevents an increase in heart/body weight ratios induced by pressure-overload hypertrophy (*23*). Abdominal aortic banding resulted in a 27% increase in heart/body weight ratios in the vehicle-treated group, whereas the CsA-treated group failed to show an increase after 6 days. Error bars are ±SEM. Coarct, aortic coarctation; Veh, vehicle-treated.

tension, which is thought to activate multiple signaling pathways leading to increased concentrations of intracellular diastolic calcium (*22*). To determine whether calcineurin signaling was involved in pressure-overload hypertrophy, we studied rats subjected to abdominal aortic banding. CsA treatment prevented the 27% increase in heart/body weight ratio characteristic of the banded, vehicle-treated group (*23*) (Fig. 3).

The successful treatment of hypertrophic animal models with CsA and FK506 suggests a novel therapy for certain forms of human heart disease. However, there is an extensive clinical literature describing adverse side effects associated with long-term CsA therapy, hence new calcineurin inhibitors may need to be developed. Cardiac transplant patients who receive CsA therapy, for example, suffer from nephrogenic toxicity and hypertension (*24*). However, clinical trials performed to date have not conclusively examined a correlation between CsA and a benefit to patients with various forms of heart disease (*25*).

References and Notes

1. B. J. Maron, J. M. Gardin, J. M. Flack, S. S. Gidding, D. Bild, *Circulation* **92**, 785 (1995); W. T. Abraham and M. R. Bristow, *ibid.* **96**, 2755 (1997).
2. T. M. Olson, V. V. Michels, S. N. Thibodeau, Y.-S. Tai, M. T. Keating, *Science* **280**, 750 (1998); K. L. Vikstrom and L. A. Leinwand, *Curr. Opin. Cell Biol.* **8**, 97 (1996); H. Watkins, J. G. Seidman, C. E. Seidman, *Hum. Mol. Genet.* **4**, 1721 (1995); B. J. Maron, *Lancet* **350**, 127 (1997).
3. K. A. Palmiter and R. J. Solaro, *Basic Res. Cardiol.* **92**, 63 (1997).
4. J. D. Molkentin *et al.*, *Cell* **93**, 215 (1998).
5. R. E. Dolmetsch, R. S. Lewis, C. C. Goodnow, J. I. Healy, *Nature* **386**, 855 (1997); P. De Koninck and H. Schulman, *Science* **279**, 227 (1998).
6. M. A. Sussman *et al.*, *Circ. Res.* **82**, 94 (1998); M. A. Sussman *et al.*, *J. Clin. Invest.* **101**, 51 (1998).
7. TOT mice develop signs of cardiomyopathy by 11 to 12 days after birth. CsA (15 mg/kg of body weight, twice a day) or FK506 (3 mg/kg twice a day) treatment was begun 9 days after birth and continued until day 24. Injections were administered subcutaneously. By day 24, vehicle-treated TOT mice showed a prominent dilated heart phenotype, whereas CsA- and FK506-treated TOT mice did not develop a pathologic phenotype. Nontransgenic mice were treated with CsA over this same period to control for effects on normal developmental cardiac hypertrophy. At least five genetically identical mice were tested within each group. For morphometric measurements, see *Science* Online (www.sciencemag.org).
8. Cryosections of hearts from TOT mice and nontransgenic mice were prepared for α-actinin and tropomodulin immunohistochemistry (*6*). The sections were generated from hearts subjected to the treatment regimens described in (*7*).
9. H. W. Lim and J. D. Molkentin, data not shown.
10. G. Wiederrecht, E. Lam, S. Hung, M. Martin, N. Sigal, *Ann. Acad. Sci.* **696**, 9 (1993); N. A. Clipstone, D. F. Fiorentino, G. R. Crabtree, *J. Biol. Chem.* **269**, 26431 (1994); H. Husi, M. A. Luyten, M. G. Zurini, *ibid.*, p. 14199.
11. Calcineurin phosphatase activity was assayed with a [^{32}P]adenosine triphosphate–labeled phosphopeptide in the presence or absence of a specific calcineurin autoinhibitory peptide. The conditions of the assay were as described [A. Gualberto *et al.*, *J. Biol. Chem.* **273**, 7088 (1998)].
12. M. C. Schaub, M. A. Hefti, B. A. Harder, H. M. Eppenberger, *J. Mol. Med.* **75**, 901 (1997).
13. J. G. Fewell *et al.*, unpublished data.
14. The MLC2v transgenic mice gradually present with a hypertrophic phenotype by 8 weeks of age. These mice were split into two groups and treated with vehicle or CsA (15 mg/kg twice a day) beginning at 2 weeks after birth and continuing through 8 weeks. Nontransgenic littermate mice were analyzed in parallel to show normal heart morphology. For heart/body weight ratio measurements, see *Science* Online (www.sciencemag.org).
15. M. Muthuchamy, G. P. Boivin, I. L. Grupp, D. F. Wieczorek, *J. Mol. Cell. Cardiol.*, in press; M. Muthuchamy *et al.*, *J. Biol. Chem.* **270**, 30593 (1995); K. A. Palmiter, Y. Kitada, M. Muthuchamy, D. F. Wieczorek, R. J. Solaro, *ibid.* **271**, 11611 (1996).
16. Overexpression of the β-tropomyosin fetal isoform in transgenic mouse hearts results in dilated cardiomyopathy by 10 days after birth. Transgenic and nontransgenic mice are phenotypically indistinguishable at 3 days after birth. The two groups were treated with CsA (15 mg/kg twice a day) beginning on day 2 after birth and continuing through day 10, at which time the untreated transgenics began to die from severe cardiac dilation.
17. M. C. Colbert *et al.*, *J. Clin. Invest.* **100**, 1958 (1997).
18. Transgenic mice expressing an activated RAR in the heart show phenotypic signs of hypertrophy by 8 weeks of age. CsA was administered as in (*14*).
19. J. Y. Le Guennec, E. White, F. Gannier, J. A. Argibay, D. Garnier, *Exp. Physiol.* **76**, 975 (1991); K. Hongo, E. White, C. H. Orchard, *Am J. Physiol.* **269**, C690 (1995).
20. A. Rao, C. Luo, P. G. Hogan, *Annu. Rev. Immunol.* **15**, 707 (1997).
21. J. James and J. Robbins, *Am. J. Physiol.* **273**, H2105 (1997).
22. L. S. Maier, R. Brandes, B. Pieske, D. M. Bers, *ibid.* **274**, H1361 (1998).
23. Six female Sprague-Dawley rats (200 to 225 g) were used for each treatment group. CsA (20 mg/kg once a day by intraperitoneal injection) was begun 2 days before surgical banding of the abdominal aorta above the renal arteries or sham operation. Treatment was maintained for six additional days, at which time the experiment was terminated because of lethality in the banded, CsA-treated group of rats. Lethality was presumably attributable to the lack of an adequate hypertrophic response that normally compensates for reduced blood flow below the level of an aortic constriction.
24. H. O. Ventura, F. S. Malik, M. R. Mehra, D. D. Stapleton, F. W. Smart, *Curr. Opin. Cardiol.* **12**, 375 (1997).
25. A. Haverich, A. Costard-Jackle, J. Cremer, G. Herrmann, R. Simon, *Transplant Proc.* **26**, 2713 (1994).

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